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15. The values of NAT prior to culture were 2.72 nmole per pineal gland per hour for control glands from chickens killed in the light; placing the glands in organ culture resulted in initial culture control NAT of 2.66 nmole per pineal gland per hour—not a significant difference. The value of NAT prior to culture of control glands from chickens killed in the dark was 25.69 nmole per pineal gland per hour (9.4 times the light time value); placing the dark time glands in culture reduced the NAT to 10.35 nmole per pineal gland per hour, a loss of 60 percent of the activity. We know from previous work [S. Binkley, J. Riebman, K. Reilly, *Comp. Biochem. Physiol.*, in press] that dark time NAT is unstable and attribute the loss going into culture to that instability. The values for the controls are averages for the 11 "light-killed" experiments (44 control animals) and the 4 "dark-killed" experiments (16 control animals).
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r and K Selection in Experimental Populations of *Escherichia coli*

Abstract. *Populations were adapted in vitro under density-dependent and density-independent population controls. Comparison of strains fails to demonstrate any trade-off in adaptation under these population controls.*

Although early efforts in ecology and population dynamics have been concerned principally with identifying and describing the action of population controls, more recent studies have sought to relate those controls to particular species' life history features. The most prominent such theory terms the action of population controls on species characters as r or K selection.

The term r selection refers to selection exerted by a density-independent type of population control under which growth is continuous and the population infrequently or never reaches its limit (*1*). This is thought to selectively favor an increased growth rate, a reduced body size, and reduced interspecific competitive ability. The term K selection refers to the effects of density-dependent regulation where the population is usually at its saturation density and seldom undergoes prolonged periods of growth. Natural selection here promotes a reduced growth rate, increased body size, and greater efficiency in competition and the use of resources.

Although this hypothesis makes numerous explicit predictions regarding the effects of these types of selection on particular life history features, the most fundamental provision of this theory is that adaptation under r and K selection results in a trade-off. That is, the effects of selection are opposed: Adaptation to K selection reduces fitness or effectiveness under the conditions in which r selection occurs and vice versa.

Since unpredictable weather is thought to function as a density-independent control, the effects of r and K selection are assumed to be continuously distributed along climatic gradients, with K selection (density-dependence) predominating in stable climates and r selection (density-independence) predominating where climate is harsh and unpredictable. Tests of this hypothesis, therefore, have often consisted of extensive comparisons of life history features in populations distributed along climatic or latitudinal gradients (*2*).

To determine whether populations are controlled dependently or independently of density is difficult, however, at best (*3*). Such studies, therefore, usually fail to attribute the observed demographic features with surety to either type of control. Nevertheless, the terms r selected or K selected are often applied to species simply because their life history features fit the expected pattern (*4*).

I report here a direct experimental test of this theory. In this study, r and K selection are applied to experimental populations of prokaryotic organisms in controlled environments, and adaptations in life history features under these controls

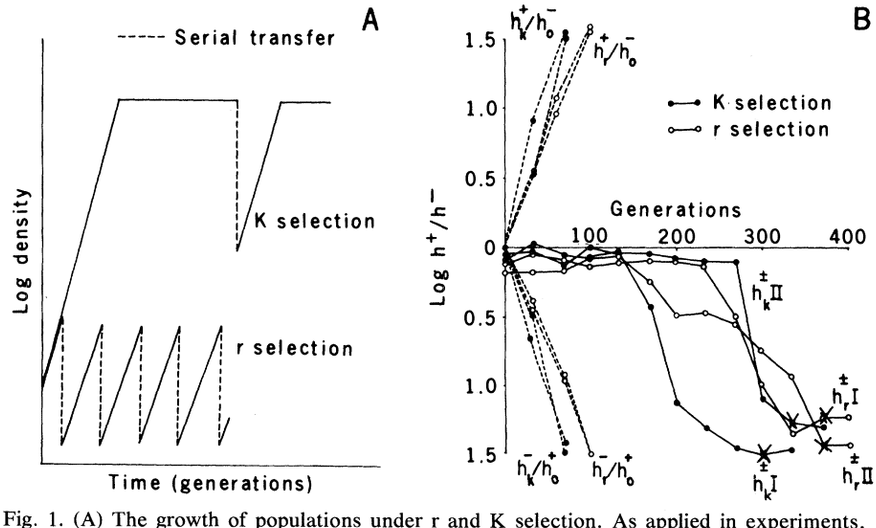

Fig. 1. (A) The growth of populations under r and K selection. As applied in experiments, populations under density-independent control are rarefied by serial transfer before reaching high density. Under density-dependent control, populations are usually at their limit. (B) The ratio of markers (h^+/h^-) diverges as strains adapt under r and K selection. Crosses (×) indicate the point at which adapted strains were isolated and repurified from populations under selection. Rapidly diverging ratios of h^+/h^- (dashed lines) show the superiority of adapted strains ($h^{\pm}_K$ or $h^{\pm}_r$) in competition with parental strains (h^+_0).

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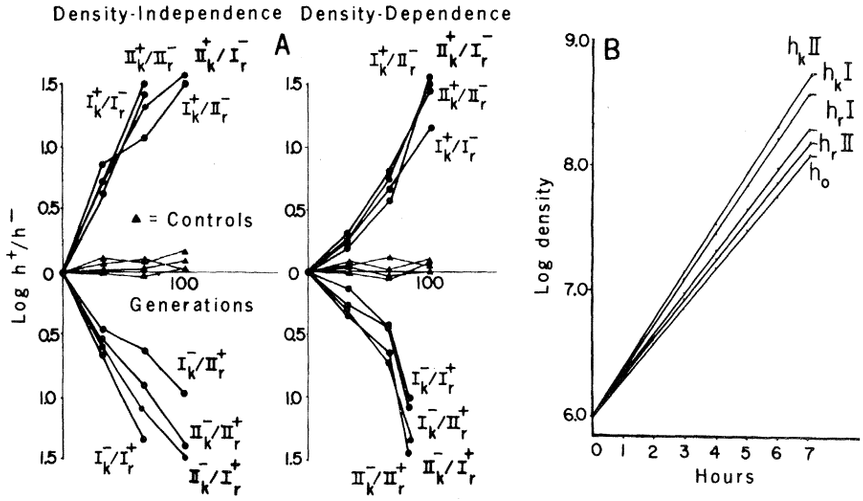

Fig. 2. (A) The growth in mixed culture of all pairwise combinations of r- and K-adapted strains ($h^{\pm}_{r}$I and II and $h^{\pm}_{K}$I and II). Ratios of h^{+}/h^{-} diverge in favor of the K-adapted strain under both density-dependent and independent control. Controls for experiments consisted of the following strain combinations: $h^{+}_{K}I/h^{-}_{K}I$, $h^{+}_{K}II/h^{-}_{K}II$, $h^{+}_{r}I/h^{-}_{r}I$, and $h^{+}_{r}II/h^{-}_{r}II$. (B) The growth to saturation density in separate culture of all adapted strains.

are compared. The use of this system provides both an unambiguous demonstration of the type of population controls applied and a clear observation of their effects of life history features.

Escherichia coli (American Type Culture Collection strain 15999) was selected for use in these experiments. This widely studied noncrossing strain produces auxotrophic (h^{-}) and prototrophic (h^{+}) markers for histidine that are competitively equivalent or neutral when grown in unshaken batch cultures at 37°C. Studies (*5, 6*) of periodic selection in this strain showed that the proportion of neutral markers provides an indicator of whether adaptation to culture conditions has occurred. That is, the ratio of marker-bearing bacteria (h^{+}/h^{-}) remains constant in experiments at any set value for 200 to 400 generations. Eventually, however, that ratio changes as a result of the emergence of a new mutant in growth rate. This mutant replaces all others, thus altering the frequency at which marker mutations were originally fixed. Selection, in the form of density-independent or density-dependent population control, was applied in experiments here to mixed populations of h^{+} and h^{-} until the ratio of markers was observed to change. Strains were then tested for adaptation by competing them with nonadapted parental strains. Starting ratios of auxotrophic markers were skewed in experiments in favor of h^{-}, permitting adaptation to occur first in that strain. Both h^{+} and h^{-} could then be isolated from the adapted population.

The proportion of markers in experiments was determined by dilution plating on agar with and without histidine. The complete growth medium and methods used here are essentially those of Ryan and Schneider (*6*). The concentration of glucose (0.05 percent) limits population growth in this medium.

As shown in Fig. 1A, under density-independent control, populations were subcultured (serially transferred) at intervals that permitted no more than two or three generations of continuous growth in the same medium. Samples (0.1 ml) of these bacteria will expand over 6.56 generations to their limit in 10 ml of medium in approximately 6.5 hours. Spectrophotometric measurement (550 μm) of cultures under selection allowed serial transfers to be made before populations exceeded an optical density of 0.05 (absorbance). This limited the numbers of bacteria to 5 to 6 percent of their potential population density. These cultures were refrigerated nightly at 8°C. Under r selection then, small populations underwent sustained log phase growth, free of any density-dependent limitation.

Density-dependent populations were allowed to reach and remain at their limits. The 1.0-ml samples expanded over 3.25 generations to their maximum density in less than 3 hours, where populations remained for another 9 to 10 hours before being serially transferred again. Thus, these cultures did undergo a brief expansion, but grew and existed principally under conditions of severe glucose limitations.

Figure 1B shows the adaptation of strains under r and K selection (solid lines) and the competition of adapted strains with parental strains (dashed lines). During selection the ratio of h^{+} to h^{-} is constant at first, but then diverges as adaptive mutants replace nonadapted parental markers after 200 to 400 generations. Adapted strains rapidly replace parental strains in competition. K-adapted strains are designated $h^{\pm}_{K}$I, II and r-adapted strains as $h^{\pm}_{r}$I, II. Nonadapted parental strains are designated $h^{\pm}_{0}$.

To test for the predicted trade-off in adaptation here, combinations of h^{+}_{K} and h^{-}_{r} or h^{-}_{K} and h^{+}_{r} were placed under both density-dependent and independent controls, and the ratio of h^{+} to h^{-} was observed for 60 to 100 generations. If such a trade-off exists, then, under density-independent control, r-adapted strains ($h^{\pm}_{r}$) should prove superior to K-adapted strains ($h^{\pm}_{K}$). Under density-dependent control the opposite should result.

Figure 2A shows the results of growth in mixed culture of adapted strains under density-dependent and independent controls. The K-adapted strains are clearly superior competitors under both types of population control.

Experiments were next performed to show whether the advantage of $h^{\pm}_{K}$ results from a higher growth rate or from the secretion of substances that inhibit the growth of r strains. To show this, h^{-}_{r}I and h^{-}_{r}II strains were each grown in 5.0 ml of growth medium to which had been added 3.0 ml of filtrate (0.45-μm filter) from h^{-}_{K}I or h^{-}_{K}II cultures. Growth of these strains in medium containing filtrates from h^{-}_{r} populations served as controls. Filtrates were tested from populations at their maximum density (as under density-dependent control) and at low density (as under density-independent control). Thus, inhibitory effects of h^{-}_{K} were tested under both density-dependent and independent controls.

These tests indicated (not shown) that, at high density, $h^{\pm}_{K}$ secretes an inhibitory substance (or substances) which restricts the growth of r-adapted strains. At low population numbers under density-independence, however, no inhibition occurred.

Figure 2B compares the growth to maximum density of adapted strains. Adaptation to K selection produces both a higher growth rate and saturation density (K) in the strains. The higher growth rate of these strains accounts for their superiority under density-independent control. These results suggest that K strains would be superior to r strains even if inhibitors were not produced.

In summary, mixed populations of competitively neutral markers were exposed to density-independent or density-

dependent population controls for 400 generations. Adaptation occurred, as shown by the superiority of adapted strains over nonadapted parental strains. And finally, K-selected strains are superior under both density-dependent and independent population controls. Thus, no trade-off in adaptation to r and K selection occurred here.

In the hypothesis, predicted life history features are determined solely by the density-dependent or independent status of controlling factors. These experiments are valid then, to the extent that they accurately simulate population controls.

The growth of experimental populations under density-independent control closely fits the circumstances for r selection (*1*). Populations under density-dependent control did undergo a brief initial period of unlimited growth but most of the growth occurred under strong density-dependent limitation. Therefore, although selection under this regime may not be perfect, it is predominately density-dependent and clearly qualifies as K selection.

Many other questions may be raised in relation to specific genetic mechanisms involved in adaptation in this system. In particular, how do the differences between the types of selection applied account for the differences observed in adaptive mutants? Answers to this question and others are necessary to a complete understanding of this system.

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Calcium Entry Leads to Inactivation of Calcium Channel in *Paramecium*

Abstract. *Under depolarizing voltage clamp of* Paramecium *an inward calcium current developed and subsequently relaxed within 10 milliseconds. The relaxation was substantially slowed when most of the extracellular calcium was replaced by either strontium or barium. Evidence is presented that the relaxation is not accounted for by a drop in electromotive force acting on calcium, or by activation of a delayed potassium current. Relaxation of the current must, therefore, result from an inactivation of the calcium channel. This inactivation persisted after a pulse, as manifested by a reduced calcium current during subsequent depolarization. Inactivation was retarded by procedures that reduce net entry of calcium, and was independent of membrane potential. The calcium channel undergoes inactivation as a consequence of calcium entry during depolarization. In this respect, inactivation of the calcium channel departs qualitatively from the behavior described in the Hodgkin-Huxley model of the sodium channel.*

The calcium ion performs several important functions in the ciliate *Paramecium* (*1-3*). Calcium carries the inward current during electrical excitation (*2-5*) through channels in the surface membrane covering the cilia (*6, 7*). Within the cilium, the Ca ion activates a reorientation of axonemal movement, causing the cilia to beat vigorously in reverse (*8*). Step depolarizations under voltage clamp are accompanied by characteristic membrane currents—an initial inward current, carried by Ca^{2+} (*5, 9*), which quickly relaxes (that is, decays) and is followed under moderate and large depolarizing pulses by a delayed outward current attributed to an efflux of K^+. Relaxation of the initial inward current resembles, superficially, the inactivation (the state of insensitivity to depolarization) exhibited by the early current carried through the Na channels of nerve (*10*), which inactivate with time as a function of membrane voltage.

Relaxation of the transient inward current in *Paramecium* (*5, 9*) might result from (i) the development of an outward current that masks a noninactivating steady Ca current, (ii) a drop in the Ca equilibrium potential (E_{Ca}) produced by the entry and intraciliary accumulation of Ca, or (iii) inactivation of the Ca channel. We now provide evidence that inactivation is the major factor causal to the relaxation of the Ca current. More significant, we provide evidence that the inactivation of the Ca system of *Paramecium* does not occur as a direct result of membrane depolarization, but that it occurs as a consequence of Ca entry that follows activation of the Ca channels by depolarization.

Specimens of *P. caudatum* were impaled with current passing and recording electrodes (10 to 30 megohms) for voltage clamping. The membrane was voltage-clamped at a holding potential equal to the resting potential. The recorded resting potential in *Paramecium* is nonspecifically sensitive to changes in both monovalent and divalent cation concentration (*11*). As compared to the behavior typical of metazoan membranes (that is, squid axon) the current-voltage relations for the early inward and late outward currents in *Paramecium* characteristically shift in an amount approximating the change in the resting potential when the extracellular ionic strength is altered. Therefore, when varying extracellular Ca, Ba, and Sr concentrations, potentials were plotted (Figs. 1-3) as displacement from the holding (resting) potential rather than by the more conventional manner of plotting absolute potential. To minimize series resistance errors in the solutions of low ionic strength, an extracellular electrode (10 to 30 megohms) was placed near the cell surface. Membrane voltage steps were 90 percent complete within 1.0 msec, and the capacitive current transients were over within 1.5 msec. Specimens were bathed in a control solution of 1 m*M* $CaCl_2$, 4 m*M* KCl, and 1 m*M* Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer at *p*H 7.1. For certain experiments, the 1 m*M* $CaCl_2$ was replaced by 0.1 m*M* $CaCl_2$ plus 5 m*M* $BaCl_2$ or 0.1 m*M* $CaCl_2$ plus 5 m*M* $SrCl_2$. Some extracellular Ca is required for membrane integrity in *Paramecium*. In another experiment, a mixture of 50 m*M* CsCl plus 50 m*M* TEA-Cl (tetraethylammonium-chloride) + 10 m*M* Pipes [piperazine-*N*-*N'*-bis(2-ethanesulfonic acid)] buffer (*p*H 7.0) was introduced by iontophoresis into clamped cells, with 10^{-9}-A, 60-second current pulses.

Depolarization of 5 mV or more in

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